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Brief report
Measurement of synovial fluid volume using urea[1]

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Summary

Objective: To examine the utility of using urea concentrations for determining Synovial Fluid (SF) joint volume in effused and non-effused joints.

Methods: Knee joint SF was aspirated from 159 human study participants with symptomatic osteoarthritis of at least one knee either directly (165 knees) or by lavage (110 knees). Serum was obtained immediately prior to SF aspiration. Participants were asked to rate individual knee pain, aching or stiffness. SF and serum urea levels were determined using a specific enzymatic method run on an automated CMA600 analyzer. Cell counts were performed on direct SF aspirates when volume permitted. The formula for calculating SF joint volume was as follows: $V_j = C_D(V_l)/(C - C_D)$ with V_j = volume of SF in entire joint, C_D = concentration of urea in diluted (lavage) SF, V_l = volume of saline injected into joint, and C = concentration of urea in undiluted (neat) SF derived below where $C = 0.897(C_S)$ and C_s = concentration of urea in serum.

Results: There was an excellent correlation ($r^2 = 0.8588$) between SF and serum urea in the direct aspirates with a ratio of 0.897 (SF/serum). Neither urea levels nor the SF/serum ratio showed any correlation with Kellgren Lawrence (KL) grade, or cell count. While urea levels increased with age there was no change in the ratio. Intraarticular SF volumes calculated for the lavaged knees ranged from 0.555 to 71.71 ml with a median volume of 3.048 ml. There was no correlation of SF volume to KL grade but there was a positive correlation ($P = 0.001$) between SF volume and self-reported individual knee pain.

Conclusion: Our urea results for direct aspirates indicate an equilibrium state between serum and SF with regard to the water fraction. This equilibrium exists regardless of disease status (KL grade), inflammation (cell count), or age, making it possible to calculate intraarticular volume of lavaged joints based upon this urea method. Most of the joint volumes we calculated fell within the previously reported range for normal knees of 0.5–4.0 ml. The positive correlation between SF volume and knee symptoms reinforces the clinical utility of this method for quantifying SF volume.

Key words: Urea, Osteoarthritis, Synovial fluid, Pain.

Introduction

Synovial Fluid (SF) derived markers are often used to monitor tissue degradation in joint diseases such as Rheumatoid Arthritis (RA) and Osteoarthritis (OA) in humans and animals. Monitoring of a single index joint is most directly done by analysis of joint fluid. At times it becomes desirable to estimate SF volume to determine total amount of a biomarker in the joint. This may be of special use in cases of effusion.

Estimations of SF volume have typically been performed with radioisotopic dilution techniques that include [^{113}In]-indium chloride or sodium [^{99}Tc]-pertechnetate[1]. Nearly two decades ago, a non-radioactive method was reported for estimating intraarticular volume based upon albumin dilution upon intraarticular injection of saline[2]. This method required aspirating an initial volume of SF directly. The retained or residual volume was estimated by injecting a known quantity of saline (10–50 ml) followed by 2 min of external joint massage to promote intraarticular mixing, followed by reaspiration of the diluted SF. The concentration of albumin in this latter sample, compared to the concentration of albumin in the undiluted sample aspirated first, allowed determination of the residual volume.

Another reported method of measuring SF volume relied upon injection of high molecular weight dextrans (one fluorescently tagged) to minimize potential efflux of marker from the joint after intraarticular injection[3]. This study was limited by the need to inject a non-native molecule, and the use of a hypertonic solution to further counteract efflux of marker from the joint. In so doing however, the hypertonic solution may have promoted transsynovial fluid flux into the joint, leading to an artifactual increase in intraarticular volume. Interestingly, the intraarticular volumes determined by albumin dilution were comparable to estimations based upon proteoglycan dilution but not hyaluronan dilution. The authors posited that hyaluronan adhered to intraarticular surfaces, and thus underestimated intraarticular volumes, while albumin did not.

A more recent study estimated joint fluid volume in rabbits by dividing the total calcium concentration in the joint cavity lavage (μg/joint) by calcium concentration in the plasma (μg/ml)[4]. To confirm the relevance of the method,

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radioactivity in the plasma and joint cavity lavage was determined after intravenously injecting ^3H_2O. Based upon the calcium ratio technique, joint fluid volumes ranged from a mean 401 μl/joint in normal rabbits to 680 μl/joint in arthritic rabbits. These correlated with results obtained from the radioisotope method ($r = 0.985$). An advantage of this study was that it relied upon a native molecule, however, calcium can be taken up by cells in the various joint tissues and therefore is subject to error dependent upon the metabolic activity of the cells.

We further explored a method of determining intraarticular SF volume, based upon measuring urea, that we previously showed was useful for quantifying dilution of SF after joint lavage[5]. Urea is a small molecule (60 Da), it is neither synthesized nor metabolized by joint tissues, and it diffuses freely between the blood and intraarticular space. Maroudas has reported that urea also freely diffuses between an external solution and cartilage with a partition coefficient of 1.0 for 200−600 μm slices stirred in solute for 30 min[6]. Additionally, it has been shown that a large amount of albumin, another native molecule, resides in the periarticular tissue mass[7]. For these reasons, we also examined the potential for this method to be confounded by a contribution of urea from cartilage during the course of a brief lavage procedure. We also examined the relationship between joint volume and self-reported knee pain.

Methods

PARTICIPANTS

A total of 159 participants were enrolled in the NIH funded POP study (Prediction of Osteoarthritis Progression). Participants met American College of Rheumatology criteria for symptomatic OA of at least one knee. In addition, all participants met radiographic criteria for OA with Kellgren Lawrence[8] (KL) grade of ≥ 1 in at least one knee. Participants were excluded on the basis of bilateral knee replacement or bilateral knee OA of KL grade 4. Knee radiographs were read by two graders (VBK, GV). A total of 275 knees were included in the analysis.

SAMPLE COLLECTION

Except for replaced knees (total of 10), SF aspiration was attempted for both knees of all participants. SF was aspirated directly and without lavage (neat) from 165 knees. When direct aspiration failed to yield SF, the aspiration syringe was exchanged and 10 ml sterile saline (without preservative) was injected through the same needle, thus requiring only one needle stick per arthrocentesis for each knee assessed. The time between saline injection to aspiration never exceeded 2 min. SF cell counts were performed when volumes permitted (greater than 0.1 ml available). Then SFs were centrifuged at 3500 rpm for 10 min and the supernatant was frozen at −80°C. Immediately before the SF was aspirated, blood was obtained for serum urea assessments, and serum was frozen at −80°C until analysis.

IN VITRO CARTILAGE EXPERIMENT

Fresh cartilage surgical waste specimens were collected at the time of total knee replacement. Normal appearing full-thickness cartilage slices were harvested remote from any macroscopic lesions. Plugs (8 mm^2) were taken from this slice and embedded in a well of silicon, with the bottom and sides of the cartilage sealed and only the surface exposed. The plugs were equilibrated overnight with SF. The SF was then removed, the surface patted dry, and a ×20 dilution of SF in saline was added to the surface of the cartilage for 2 min using only enough fluid (≈25 μl) to cover the surface. This simulated the *in vivo* lavage methodology. The aqueous sample was then removed and analyzed for urea and compared with the urea concentration in the original ×20 dilution of SF.

UREA MEASUREMENTS

Concentrations of urea were determined by a CMA600 microdialysis analyzer (CMA Microdialysis, Solna, Sweden) on 5 μl samples of sera and joint fluid. Concentrations of urea were obtained by completely automated enzymatic reaction techniques. Quantification of urea in this assay depends on the rate of utilization of nicotinamide adenine dinucleotide (NAD) and was measured at 365 nm. A measurement cycle was completed in less than 2 min. All reagents were obtained from CMA microdialysis.

PAIN MEASUREMENTS

Participants with OA were recruited through the community and a rheumatology clinic. Some participants had bilateral knee symptoms ($n = 117$) and others unilateral knee symptoms ($n = 30$) at the time of sample collection. Participants were asked to rate individual knee pain, aching or stiffness that occurred on most days of any 1 month in the last year as well as most days of the present month. Pain was rated as none, mild, moderate or severe.

Results

The sample consisted of 159 participants with symptomatic knee OA during the last year in at least one knee; 79 had bilateral moderate to severe radiographic knee OA (KL grades 2−4), and of the total 275 knees evaluated, 91 were KL grades 0−1. The participants (118 females, 41 males) ranged in age from 35 to 85 years old with a mean age of 64 years (12 years SD). We obtained SF by direct aspiration from 165 knees and by lavage from 110 knees for a total of 275 knees aspirated. Knees were only lavaged when it was not possible to obtain fluid directly. Neat knee aspirations yielded on average 2.3 ml (2.7 ml standard deviation (SD)) SF and lavaged joints yielded 2.8 ml (3.3 ml SD) SF. There was an excellent correlation ($r^2 = 0.8588$) between SF and serum urea in the direct aspirates (Fig. 1) with a ratio of 0.897 (SF/serum). There was also an excellent correlation ($r^2 = 0.984$) between SF urea concentrations between paired contralateral joints aspirated directly. As expected, neither urea concentrations nor the SF/serum ratio showed any correlation with KL grade or cell count. While urea concentrations increased with age there was no change in the ratio. In our cartilage explant diffusion study we found no evidence of cartilage contributing urea during a simulated 2-min lavage procedure.

The formula for calculating SF joint volume in the lavaged joints, regardless of effusion, is a modification of the albumin dilution method[2], which uses the measurement of a native molecule before and after dilution with a known amount of saline. The volume of saline can vary as long as it is sufficient to provide adequate mixing within the joint. The formula was as follows: $V_j = C_D(V_I)/(C - C_D)$ with $V_j =$ volume of SF in entire

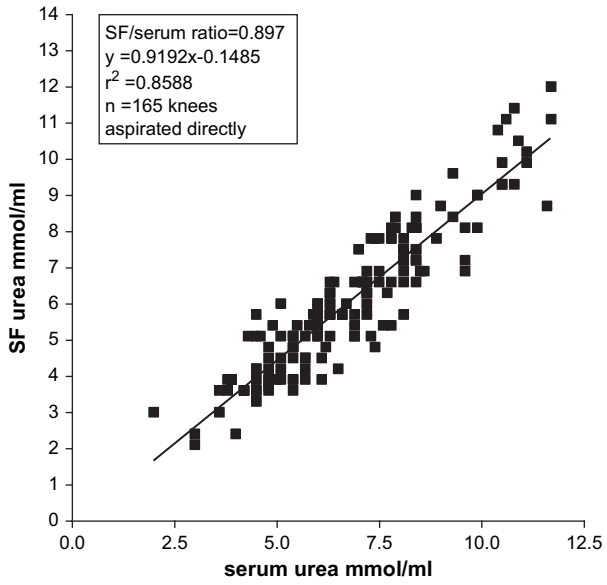

Fig. 1. Correlation of SF and serum urea concentrations (mmol/ml) in SF samples aspirated directly (without lavage).

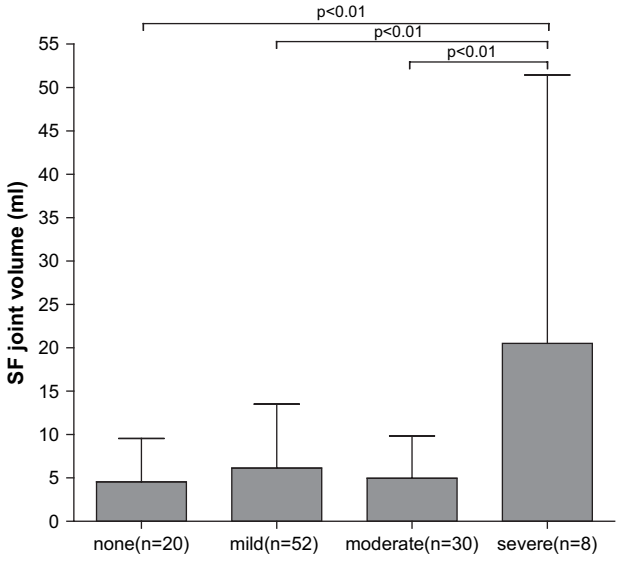

Fig. 2. Knee joint SF volume by pain in knees aspirated by lavage ($n = 110$). The mean (+SD) SF volume is shown relative to self-reported knee pain on most days of present month on a 4-point scale. Bonferroni's multiple comparison test is shown. One-way ANOVA: $P = 0.001$.

joint, C_D = concentration of urea in diluted (lavage) SF, V_I = volume of saline injected into joint, and C = concentration of urea in undiluted (neat) SF. Since our method uses only one aspiration, the concentration of analyte before dilution (C) is derived using the serum urea (C_S) as a proxy for the neat SF urea. Since the SF urea in neat samples is slightly lower but highly correlated to serum urea, C was derived from the ratio of SF/serum urea in neat samples as described above, multiplied by the concentration of urea in serum C_S: $C = 0.897(C_S)$. Intraarticular SF volumes calculated for the lavaged knees ranged from 0.56 to 71.71 ml with a median volume of 3.05 ml. There was no correlation of SF volume to KL grade but there was a positive correlation by analysis of variance (ANOVA) ($P = 0.001$) between SF volume and self-reported individual knee pain on most days of the present month (Fig. 2) as well as most days of any month in the last year ($P = 0.001$). The mean SF volume was 4.5 ml for knees without pain ($n = 20$). The mean SF volume was 7.0 ml for knees with any pain ($n = 90$). The eight knees with most severe pain were associated with the highest mean SF volume (20.5 ml).

Discussion

We observed an SF/serum urea ratio of 0.897 in direct aspirates of OA knees. In another study, the SF/serum urea ratio in RA ($N = 10$) and OA ($N = 11$) patients was 1.14 (Ilya Tchetverikov and TeKoppele, unpublished communication). Both these results are similar to the ratios of 1.06 in healthy canines[5] and 0.95 in bovines[9] with no signs of arthritis. This ratio indicates an equilibrium state between serum and SF with regard to the water fraction in RA/OA patients. This equilibrium exists regardless of disease severity (KL grade), inflammation (cell count), or age, making it possible to calculate intraarticular volume of lavaged joints based upon this urea method. This known relationship can be used to estimate intraarticular SF volume in normal joints and those with chronic arthritides.

Most of the joint volumes we calculated fell within the previously reported range for normal knees of 0.5–4.0 ml[10]. The limitations of the method include the need for adequate mixing after saline injection as well as the probable need for a minimum of 10 ml of saline for lavage. Although a previous study recommended 15 ml or more of saline per injection[2], we chose to inject 10 ml of saline as an amount that would give us good recovery while keeping participant discomfort to a minimum. This method may not be applicable in joints with acute effusions, for instance acute joint injury, as the serum to SF urea ratio may vary from what we have found for normal joints and chronically arthritic joints described here. As long as the time of lavage is kept to a minimum, urea washout from surrounding tissues should have no effect on the volume calculation. Since we did not test the efflux of urea from synovial tissue, we cannot exclude a contribution of urea from this source that would serve to result in an overestimation of intraarticular SF volume. However, we do not expect such confounding to be an issue for two reasons: first, the minimal duration of the lavage procedure; and second, our previous experience demonstrating an excellent correlation of four separate SF analytes measured in one knee from a neat sample and in the contralateral knee by the urea-corrected lavage method[5]. It is nevertheless important to keep in mind that any confounding of intraarticular volume estimates by potential contributions of urea from adjacent joint tissues would be lessened by the presence of an effusion.

The positive correlation between SF volume and knee symptoms provides independent confirmation of the recently reported association of knee pain and stiffness with moderate and large joint effusions by magnetic resonance imaging (MRI)[11]. This correlation reinforces the clinical utility of this method for quantifying SF volume. It would be valuable to further validate this method with intraarticular volume estimation by MRI.

Acknowledgments

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